PATENT
0425-0847P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	Koichi ITO et al.	Conf.:	9635
Appl. No.:	09/913,444	Group:	1624
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For:	HETERODIAZINON COMPOUND		

DECLARATION UNDER 37 C.F.R. § 1.132

I, Takahisa Hanada, declare as follows:

1. I am one of the co-inventors of the invention claimed in the above-referenced application. I have carried out comparative tests which are fully described herein, to support enablement of the invention, and to show unexpected superior properties of the compounds of the present invention over U.S. Patent 4,670,555 to Dekeyser et al.

2. A Formalin assay was conducted either by myself or under my supervision and control to show that the present invention is effective in treating pain. In the assay, the analgesic effect of the compound of claim 1, particularly compound #54 was tested. In the test, mice were anesthetized lightly with halothane. 20 μl of 3% formalin solution was injected subcutaneously into the dorsal

surface of the left hind paw of the mice. The mice were then placed in an observation chamber. Compound #54 was intravenously administered to the mice just before the formalin injection. The mice demonstrated pain related licking and biting behavior. These responses were measured during the first five minutes after the formalin injection. The example Compound #54 reduced the pain related licking and biting behavior in the mice. This result indicated that compound #54 has an analgesic effect. The results are shown in Table 1 attached hereto.

3. An EAE assay was conducted with Lewis rats. The Lewis rats were immunized s.c. in each hind foot with 25 µl of inoculum containing 50 µl MBP emulsified in Freund's complete adjuvant containing *M. tuberculosis*. Thereafter, a vehicle (methyl cellulose (MC)) or example compound #1 was administered to the rats. On day 7, post immunization, 150 and 3000 mg/kg of each were administered twice daily p.o. This administration continued up to and including day 16, post-immunization. Changes in body weight and neurological scores were monitored everyday. The results are demonstrated in Table 2 attached hereto. Compound #1 was shown to be effective in eliminating paralysis and an unstable gait in the rats. The results indicate that Compound #1 is effective in treating epilepsy.

4. Comparative *in vitro* and *in vivo* tests were conducted utilizing example compounds 1, 33, 34, 42, 54, 61, 66 and 68 listed in the specification. The *in vitro* assay was conducted in the same manner as in the specification at pages 107-108. The results of the *in vitro* assay are reported in Table 3 attached hereto. Examples 2, 5, 6, 8, 14, 27, 28 and 29 of U.S. Patent 4,670,555 to Dekeyser et al. were also tested in the same manner. The results of the *in vitro* assay are also reported in Table 3. The comparative results demonstrate that the IC50 values for the compounds of the present invention are unexpectedly superior to the compounds in Dekeyser.

An *in vivo* assay was conducted to test the above-mentioned compounds of the invention against the above-mentioned compounds of Dekeyser. The *in vivo* assay was conducted in the same manner as the *in vivo* assay described in the specification at pages 112-113. The action of the test compound was judged by the presence or absence of ~~spasms~~ seizures 1/29/2004 T.H. in the mice. A total of six mice were tested. The results are reported in Table 3 in terms of the number of mice out of 6, that actually had ~~spasms~~ seizures 1/29/2004 T.H. The results of the *in vivo* assay demonstrate that the compounds of the present invention are more effective in reducing ~~spasms~~ seizures 1/29/2004 T.H than the compounds of Dekeyser et al. These results were unexpected.

5. I hereby declare that all statements made herein of my own knowledge are true. Statements made on information and belief are believed to be true to the best of my knowledge. These statements were made with the full knowledge that willfully providing false statements and the like are punishable by fine and/or imprisonment under §1001 of Title 18 of the United States Code, and that willful false statements may jeopardize the validity of the application or any patent that may grant therefrom.

DATED: 11/29/2004 BY: Takahisa Hanada
Takahisa HANADA

Attachments: Tables 1-3

TABLE 1

Results

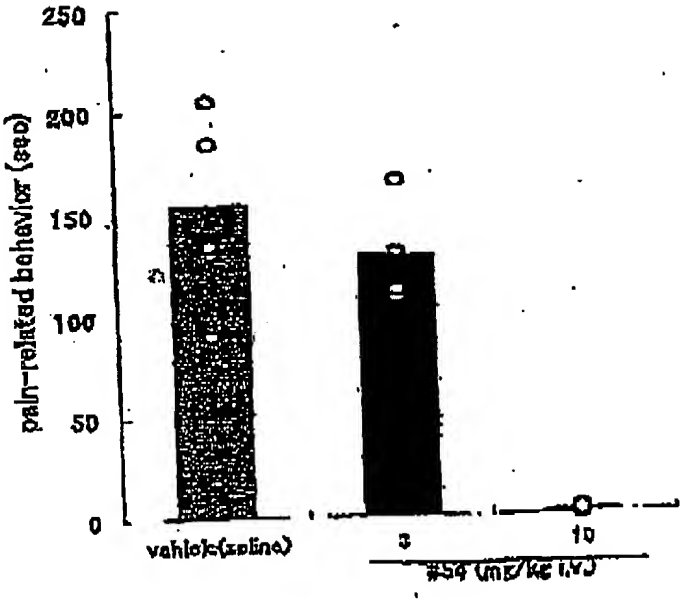


TABLE 2

The following scoring system was used to grade neurological impairment:

0:	Normal	
0.5:	Half paralyzed tail	
1:	Frazzled tail	
1.5:	Slightly unsteady gait	
2:	Unsteady gait	
2.5:	Complete paralysis of one hindlimb	
3:	Complete paralysis of both hindlimbs	
3.5:	Complete paralysis of both hindlimbs	and weakness of one forelimb
4:	Complete paralysis of both hindlimbs	and weakness of both forelimbs
5:	Moribund/death.	

Results

Effect of example #1 compound on disease symptom

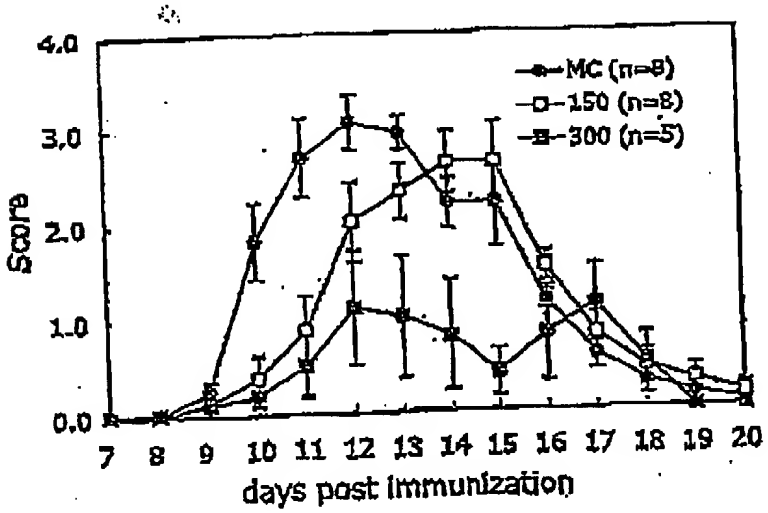
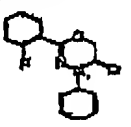
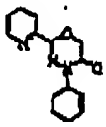
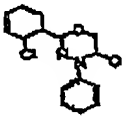
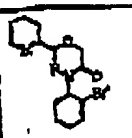
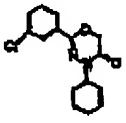
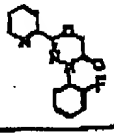
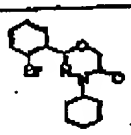
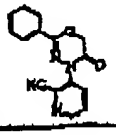
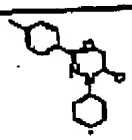
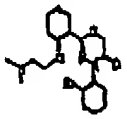
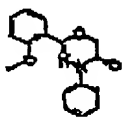
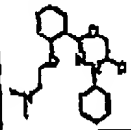
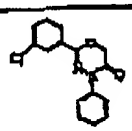
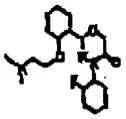
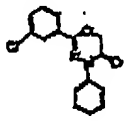
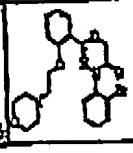


TABLE 3

Cited reference(US 4,670,555)				Our invention			
Example No.	Formula	In vitro (μM)	In vivo	Example No.	Formula	In vitro (μM)	In vivo
2		3.94	3/6	1		11.2	1/6 (*2)
5		18.9	5/6	33		4.28	0/6
8		19.4	6/6	34		9.21	0/6
8		18.8	4/4 (*1)	42		3.73	0/6
14		17.16	6/6	54		3.54	1/6
27		10.02	6/6	61		4.61	0/6
28		21.17	6/6	68		2.72	1/6
29		28.16	5/6	68		5.64	1/6

In vitro:Pharmacological experimental example 1.The value indicates IC50
In vivo:Pharmacological experimental example 8.A test compound 30mg/kg was administered to each mouse. The value indicates the number of spasmed mice in 6 mice.
(*1):15mg/kg of the compound was administered to each mouse.
(*2):10mg/kg of the compound was administered to each mouse.